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Broadband Ultraviolet CD-Spectroscopy of Ultrafast Peptide Backbone Conformational Dynamics

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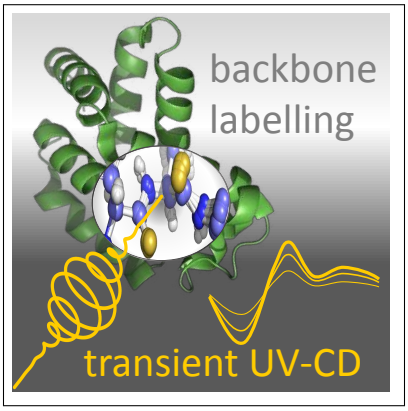
Abstract

The far UV spectral window widely used for the conformational analysis of biomolecules is not easily covered with broadband lasers. This has made it difficult to use CD spectroscopy to directly follow fast structure changes. By combining transient CD spectroscopy in the deep UV with thioamide substitution, we demonstrate a method to overcome this difficulty. We investigated a dipeptide whose two carbonyl oxygen atoms were replaced by sulfur, red-shifting the strong lowest-lying $\pi\pi^*$ transitions into the more accessible 250-370 nm spectral window. Coupling of the two thioamide units cannot be resolved by achiral 2D-UV spectroscopy, but it gives rise to a pronounced bisignate CD spectrum. The transient CD spectra reveal the weakening of this coupling in the electronically excited state, where conformational constraints are released. Our results show that direct local probing of fast backbone conformational change via CD is possible in combination with site-selective thio-substitution in peptides and proteins.

Graphical TOC Entry



One of the goals of biomolecular research is to probe the stucture of biosystems in physiological (aqueous) media and to identify structural changes in the course of functional transitions. Nuclear magnetic resonance (NMR) is a powerful technique for structure determination in solution, but its time resolution is intrinsically limited to microseconds. Small and wide angle solution X-ray scattering may be regarded as a complementary avenue.[1] Its temporal resolution has reached the 100 ps range[2,3] and, with the advent of Free electron lasers, even the few picosecond time scale in small angle scattering.[4] However, only changes of the global structure can be retrieved. As an alternative, the principles of NMR-structure resolution can be translated to shorter wavelengths[5] in order to monitor the couplings between chromophores and their evolution as a function of time. This has been the main motivation for developing broad-band non-linear spectroscopies in the infrared,[6,7] visible[8,9] and more recently the UV.[10,11] Similar to NMR, the sensitivity of these laser-based methods is linked to the presence of spectrally well-separated transitions (of comparable oscillator strength), as encountered for example in many light-harvesting systems.[12] In contrast, circular dichroism (CD) spectroscopy, which measures the absorption difference of left and right-handed circular polarized light, can easily resolve the coupling of chromophores even in the case of highly congested absorption spectra. Different excitonic transitions give rise to CD signals of different sign, which depend very sensitively on the molecular geometry.[13] CD spectroscopy has thus become a common and powerful method for analyzing the structure and stability of biomolecules.[14] For the same reason, transient CD spectroscopy can be much more sensitive to the changes in coupling and hence to changes in molecular structure than transient absorption and other achiral non-linear methods.[15] Broadband transient CD spectroscopy with femtosecond time-resolution[16–18] has only very recently been extended to cover the deep UV between 250-370 nm.[19] In this spectral region, many important compounds such as nucleobases and aromatic amino acids can be accessed. Nevertheless, the lowest-lying electronic transitions of the polypeptide backbone, which yield most direct information on the conformation of peptides and secondary structure elements in proteins, are located at

even shorter wavelengths, which can so far only be probed with narrow-band pulses.[20] In order to detect fast conformational changes of the peptide backbone with the high structure sensitivity of broadband CD spectroscopy we here use spectroscopic labelling: The substitution of a backbone carbonyl oxygen atom by sulfur leads to a significant red-shift (by more than 60 nm) of the lowest-lying $\pi\pi^*$ and $n\pi^*$ transitions of the corresponding peptide bond[21] from the far UV well into the spectral window accessible by broadband pulses.[22] When two adjacent peptide units are labeled, the excitonic coupling of essentially only two transition dipole moments gives rise to a characteristic CD-couplet,[21,23] which encodes the information on the local structure much more directly than the conventional CD signal of an entire peptide or protein, and more sensitively than the CD of a single substitution.[24] It has already been demonstrated for both α-helical peptides[25] and β-sheet motifs[26] that thio-substitution of a single peptide unit is possible without significantly perturbing the secondary structure. Furthermore, a single thio-substitution left the folding rate of β-hairpins largely unchanged.[27] More recently, methods have been developed to synthesize full-sized proteins containing one and two thioamide bonds.[28–30] It was shown that their folding properties are only little affected by these substitutions,[31,32] and the red-shifted $\pi\pi^*$ transitions could be used as efficient, site-selective quenchers of side-chain fluorescence.[33] By probing the thioamide absorption directly with ultrafast UV-spectroscopy, it should thus be possible to study backbone structure and conformational changes in a site-specific way. In this sense, the idea of thio-substitution is very similar to isotope substitution employed to obtain site-selective information on the backbone structure in vibrational CD[34] and non-linear IR-spectroscopy[35–37] but with the advantage of much larger transition dipole moments and better spectral separation of labeled and unlabeled peptide units. In this work, we establish the feasibility of probing local backbone conformational dynamics by combining transient CD spectroscopy and thio-labelling, using a simple dipeptide as a model. The molecule, *N*-thioacetyl-proline dimethyl thioamide (Fig. 1), contains two thio-substituted amide bonds. These two excitonically coupled chromophores exhibit all the spectroscopic characteristics

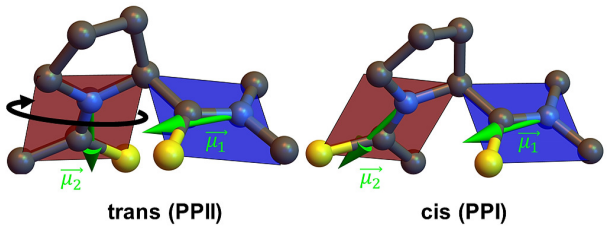


Figure 1: Trans and cis forms of *N*-thioacetyl-proline dimethyl thioamide (*L*-enantiomer). The two sulfur atoms (yellow) replace the carbonyl oxygens of the original oxopeptide for UV-labelling, as explained in the text. Green arrows indicate the two coupled $\pi\pi^*$ transition dipole moments, lying in the planes of the two thioamide bonds. They point in the direction of negative charge flow.

of a larger bi-substituted peptide or protein. In order to trigger conformational changes, we use the fact that UV-excitation leads to larger conformational flexibility in the excited state where the thioamide bond is weakened, eventually causing trans-cis isomerization upon return to the electronic ground state.[38,39] Photoisomerization of the proline moiety is expected to lead to a significant change in coupling between the two thioamide units which we probe by transient CD spectroscopy.

Site-selective peptide labelling by thio-substitution may also be an interesting tool in combination with multidimensional spectroscopy, which uses the same ultrashort broadband laser pulses in the deep UV.[10,11] Hence, in a complementary set of measurements we address the question in how far 2D-UV spectroscopy can resolve the coupling between the two adjacent thioamide units, which is also at the origin of the CD signal of the thiopeptide.

Fig. 2a shows the steady-state UV absorption spectra of *N*-thioacetyl-proline dimethyl thioamide (from now on simply referred to as thiopeptide) and of the unsubstituted parent *N*-acetyl-proline dimethyl amide (from now on referred to as oxopeptide). The main absorption peak observed in each spectrum arises from the lowest-lying $\pi\pi^*$ transitions of the amide bonds which are significantly lower in energy in the thio-substituted molecule. Due to thio-substitution the absorption maximum shifts from 198 nm to 270 nm directly into the spectral window accessible by broadband deep-UV probe pulses.[10,19] In order to overlay the spectra

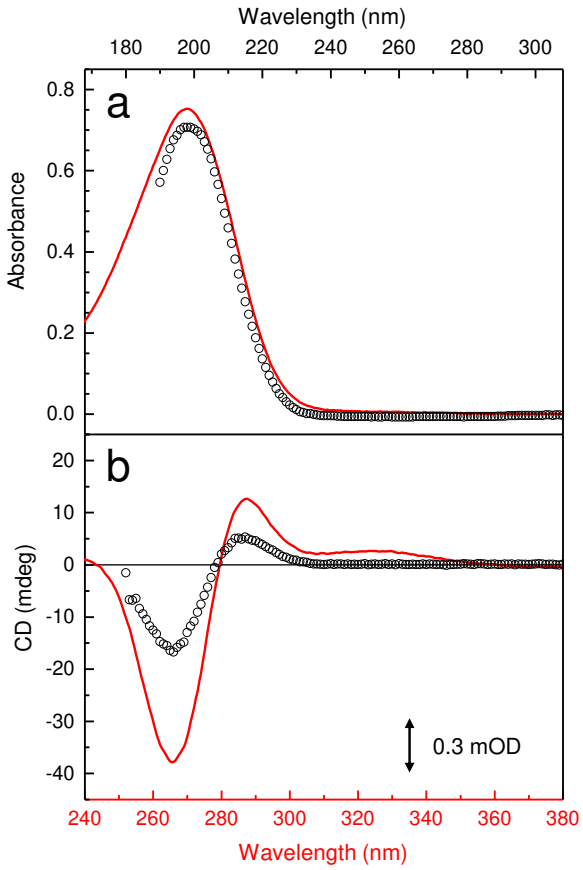


Figure 2: (a) Steady-state UV absorption and (b) stationary UV CD spectra of *N*-thioacetyl-proline dimethyl thioamide (red solid lines, bottom wavelength scale) and unsubstituted *N*-acetyl-proline dimethyl amide (circles, shifted wavelength scale on top). Spectra were recorded at room temperature for the *L*-enantiomers in H_2O.

in Fig. 2, the wavelength scale of the oxopeptide was shifted by 72 nm. Fig. 2b shows the corresponding CD spectra of the thio- and oxopeptides. Coupling of the two (thio-)amide $\pi\pi^*$ transition dipoles gives rise to a pronounced bisignate couplet. The CD signature of the thiopeptide is essentially a red-shifted copy of the oxopeptide signal. However, while the optical density of the two samples was almost identical, the amplitude of the thiopeptide CD-spectrum is significantly larger. Qualitatively, this can be explained within a simple transition dipole coupling model,[40] where the CD-signal is proportional to the cross-product $\vec{\mu}_1 \times \vec{\mu}_2$ of the individual $\pi\pi^*$ transition dipole moments and the coupling constant $\beta \propto (\vec{\mu}_1 \cdot \vec{\mu}_2)$. In fact, the $\pi\pi^*$ transition dipole moment $\vec{\mu}_i$ of a single thiopeptide bond is greater than that of an oxopeptide bond, causing a larger coupling constant (in Fig. 2 the

thiopeptide concentration was approximately half of the oxopeptide concentration). The positive signal in the thiopeptide CD spectrum at 330 nm is due to the nπ^* transition of the thiopeptide bond, which is only magnetically allowed (transition dipole moment $\vec{m}_i$) and hence not visible in the absorption spectrum. Within the dipole coupling model, its intensity in the CD spectrum arises from interaction with the $\pi\pi^*$ electronic transition dipoles and is proportional to $\beta(\vec{m}_j \cdot \vec{\mu}_i)$. Its contribution is thus expected to be weaker in the oxopeptide signal, as observed in Fig. 2b.

In the past, the oxopeptide parent molecule has served as a simple model of poly-proline structures. It was used for understanding peptide conformation near proline residues and for testing the structure-sensitivity of numerous experimental methods and theoretical predictions.[41–43] Essentially, the molecule can only adopt two structures in solution, which are characterized by the trans and cis configuration of the *N*-acetyl-proline bond (Fig. 1). Primarily due to steric interactions, the trans to cis isomerization of this bond can be considered as the only conformational degree of freedom.[41,42] In water, the equilibrium between the two conformers is dominated by the trans isomer with dihedral angles corresponding closely to those characteristic for poly-proline II helices. Less than 25% of the oxopeptide molecules adopt a structure similar to all-cis polyproline I.[41] Likewise, the ^{1}H-NMR spectrum of the thiopeptide in water also yields a cis-population of ≈20% at room temperature (see Supporting Information). The similarity of the CD spectra and isomer ratios indicates that the original peptide structure is only minimally perturbed by the thio-substitution, and the thiopeptide conformation in the electronic ground state is thus well defined with a narrow distribution of dihedral angles. Similarly well-defined initial angles can be expected for two thioamide chromophores that are part of a secondary structure motif.

For time-resolved experiments the sample solutions (2-3 mM in water) were circulated by a micro annular gear pump driving a wire-guided liquid jet (thickness ≈200 μm) to avoid contributions of the window material. The use of flow cells or thicker jets is, however, possible when concentrations need to be lower. The 2D-UV and transient CD setups have been

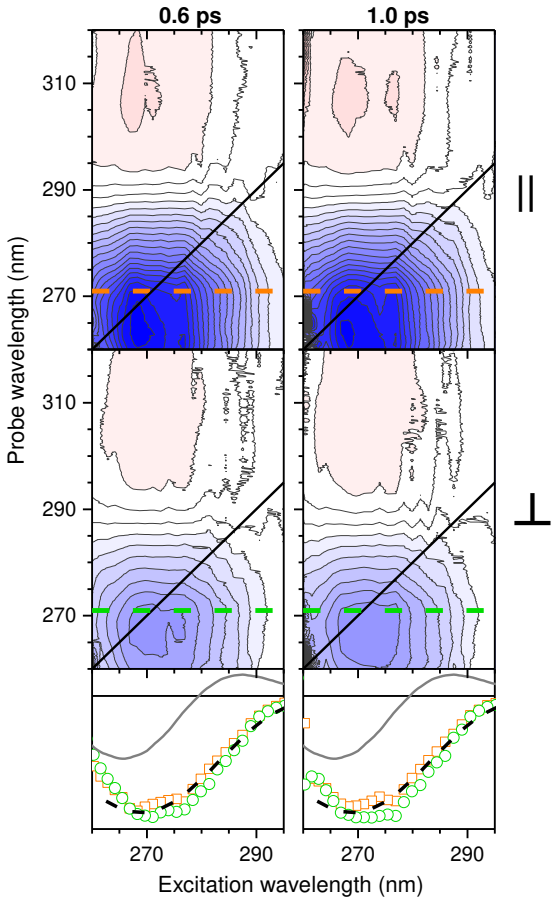


Figure 3: 2D-UV spectra of the thiopeptide at 0.6 ps (left) and 1 ps (right) time delays between parallel (top) and perpendicular (below) polarization of pump and probe pulses. Contour spacing 0.55 mOD. The diagonal is indicated by a black line. Cuts through the 2D spectra at 270 nm probe wavelength are plotted at the bottom, together with a scaled CD (solid gray) and inverted absorption spectrum (dashed). Cuts for perpendicular polarization (green) were multiplied by 2.35 to superimpose with the cuts for parallel polarization (orange).

described elsewhere[19,44] and a summary is provided in the Supporting Information. UV-excitation of the thiopeptide leads to a reduced absorption in the region of the $\pi\pi^*$ band near 270 nm (ground state bleach, GSB) and a broad induced absorption of excited molecules at wavelengths longer than 290 nm. These absorption changes are shown as a function of excitation wavelength in the 2D-UV spectra in Fig. 3 for parallel and perpendicular polarization of pump and probe pulses. The bleach region of the spectra (negative absorption change, blue) contains information about the molecule in the electronic ground state before

excitation. Since the $\pi\pi^*$ band is due to two (coupled) electronic transitions, as evidenced by the steady state CD spectrum in Fig. 2b, we may expect to see a correlation between the excitation wavelength and the minimum of the bleach signal: the higher energy transition should be preferentially excited at the shorter wavelengths while the lower energy transition should be excited at longer wavelengths, leading to two diagonally shifted bleach minima or a bleach signal that is tilted in the direction of the diagonal of the 2D-spectrum.[8,45] In contrast, we observe that the bleach minimum is independent of excitation wavelength. Neither can the two transitions underlying the $\pi\pi^*$ band be resolved by comparing the spectra recorded with parallel and perpendicular polarization of excitation and probe pulses. The horizontal cuts through the bleach region of the two sets of 2D-UV spectra in Fig 3 can be superimposed. There is thus no clear signature of a possible cross-peak between the two trar nsitions, which should have a very different polarization-dependence than the diagonal peaks.[35] Only a slightly larger shoulder of the perpendicular cuts at longer wavelengths (after normalization at 265 nm) might be interpreted as the manifestation of a second, unresolved transition. We conclude that the two $\pi\pi^*$-bands must be very close in energy and/or possess very different oscillator strength, which makes them difficult to distinguish by 2D-UV spectroscopy.

The positive signal (red) in the 2D spectra in Fig. 3 is due to excited state absorption (ESA). Its long wavelength tail >350 nm has already been reported in transient visible experiments for a series of compounds containing a single thioamide bond.[38,39] The ESA rises immediately after UV excitation and decays on a dual timescale with a fast component of ≈2 ps and a slow component of ≈80 ps (Figure S3). According to previous work, the fast timescale of the ESA decay corresponds to the relaxation to the lowest singlet state S_1 and possibly an intersystem crossing to the lowest triplet state T_1.[38,39] In both S_1 and T_1 the thioamide C-N bond is elongated and there is no significant barrier to rotation about this bond.[38] Upon return to the electronic ground state, long-lived trans to cis isomerization (and vice-versa) can take place.[46] In the thiopeptide studied here, this is limited to the

N-thioacetyl-proline bond (red peptide plane in Fig. 1), because of the dimethyl symmetry at the C-term. However, we observe isomerization only for a very small fraction of the molecules, while the vast majority returns to the original conformation. This is reflected by an almost complete decay of the transient absorption changes 1.4 ns after excitation (gray trace in Fig. 4b). It is much smaller than the residual transient signal we measured for N-methyl thioacetamide (see supporting information) where the isomerization yield is 30-40%.[38] Because of the lack of significant permanent isomerization, we concentrated our efforts on recording transient CD spectra at a few selected time delays after excitation when the molecule is still in an excited state.

Fig. 4a shows the static (unpumped) CD spectra of the *L* and *D*-enantiomers of the thiopeptide recorded with our broadband time-resolved CD-spectrometer. The signals are mirror images for the two enantiomers and almost perfectly reproduce the data recorded with a commercial spectrometer. Minor baseline distortions can be seen only at longer wavelengths. They can be ascribed to a residual asymmetry in the circular polarization of the probe caused by birefringence of the photoelastic modulator (PEM) and imaging optics in the probe path.[19] The sensitivity of the transient measurements is much higher due to pulse-to-pulse subtraction of the CD signals with and without excitation, which cancels many of the residual asymmetries. The resulting CD changes are shown for delay times of 5 and 25 ps in Fig. 4c. The signal size ($\approx 20\mu$OD) is only a few percent of the static CD and the transient absorption (Fig. 4b) and on the order of the baseline stability of the setup. In order to exclude artifacts[47,48] it is therefore important to compare the spectra for the *D* and *L*-enantiomers, which are represented by red and blue lines. Both show distinct transient CD signals peaking near 270 nm and 285 nm, with opposite sign for the differently-handed molecules. Their shape is very different from the corresponding absorption changes, making contributions from linear polarization artifacts very unlikely. Clearly, we are detecting very small ultrafast changes of the CD spectrum of the thiopeptide backbone, which apparently decay on a similar time scale as the electronically excited state. Indeed, for such a small

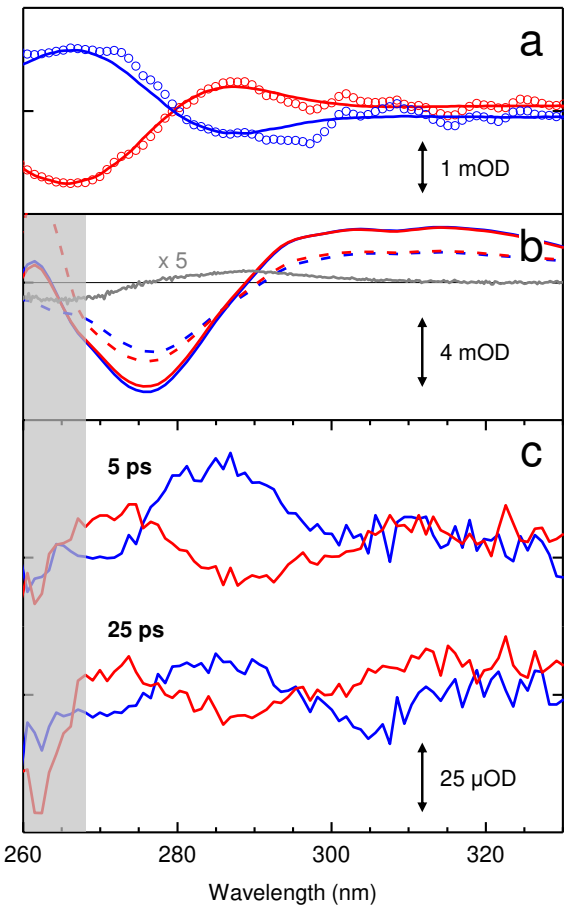


Figure 4: (a) Static CD spectra recorded with the transient CD setup (circles) and a commercial CD spectrometer (solid lines). (b) Transient absorption changes 5 ps (solid lines), 25 ps (dashed lines) after UV-excitation at 266 nm. (c) Corresponding transient CD spectra. Red: *D*-enantiomer, blue: *L*-enantiomer. The gray area in (b) and (c) marks the spectral region with contributions from pump-light scattering, the gray line in b) shows an enlarged view of the tiny transient absorption signal remaining after 1.4 ns delay (see SI for a full set of transient spectra).

molecule, it is unlikely that the kinetics observed by CD are significantly different from transient absorption during electronic relaxation.

The transient CD signals in Fig. 4 qualitatively look like mirror images of the static CD spectra with a common zero-crossing point near 280 nm. The peak near 285 nm appears to be relatively stronger in the transient CD spectra, but it is likely that scattered pump light distorts the data below 270 nm (gray region in Fig. 4). Also, the transient CD spectra could not be corrected for the relatively large chirp of the probe pulses.[19] An important fraction

of the transient CD signal may thus be attributed to the depopulation of the electronic ground state. Thanks to the very high sensitivity of our set-up, we were able to record this small change of the backbone CD signal despite the fact that the thiopeptide showed very little long-lived isomerization, which would have resulted in a much larger change in coupling between the two thioamide units. At the same time, this indicates that UV-induced isomerization of the thiopeptide bonds will not contribute strongly to the signal when conformational change is triggered by other means. The coupling of two spectrally and spatially isolated transition dipoles and its change due to structural change can be much better resolved by broadband CD than by linear or non-linear absorption techniques, and the change in signal can be directly related to a change in local dihedral angles. Our next goal is to use two coupled thioamide units no longer for photo-triggering, but exclusively as a local probe of conformational dynamics in larger peptides or even proteins. Much larger structural rearrangements can be induced, for example, by laser-triggered pH or temperature jumps.[49,50] The resulting transient CD-signals should not only be much more pronounced but also free of scattering contributions, when the excitation wavelength is no longer within the deep-UV probe window.

We have demonstrated that two important conditions can be met in order to directly probe the backbone conformation of peptides and proteins undergoing ultrafast structural change. Firstly, the substitution of two neighboring carbonyl-oxygen atoms by sulfur in the peptide backbone can be used as a site-selective spectroscopic label with a very structure-sensitive CD signal, far away from the bulk protein absorption and well-accessible by broad band UV laser pulses. Secondly, the sensitivity of our time-resolved CD spectrometer, and - equally important - the suppression of artefacts, is sufficient to detect even very small changes of this backbone signal with picosecond time resolution. In view of recent advances in introducing pairs of thioamide labels in proteins,[30,32] we are confident that this approach will become very useful in probing local structural changes in fast folding studies as well as the backbone response to light-stimulation of photosensitive proteins.

Acknowledgement

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Supporting Information Available

Experimental details, NMR spectra of the studied compounds, transient absorption spectra.

References

(1) Hub, J. S. Interpreting solution X-ray scattering data using molecular simulations. *Curr. Opin. Struct. Biol.* **2018**, *49*, 18–26.

(2) Cammarata, M.; Levantino, M.; Schotte, F.; Anfinrud, P. A.; Ewald, F.; Choi, J.; Cupane, A.; Wulff, M.; Ihee, H. Tracking the structural dynamics of proteins in solution using time-resolved wide-angle X-ray scattering. *Nat. Methods* **2008**, *5*, 881–886.

(3) Kim, K. H.; Muniyappan, S.; Oang, K. Y.; Kim, J. G.; Nozawa, S.; Sato, T.; Koshihara, S. Y.; Henning, R.; Kosheleva, I.; Ki, H. et al. Direct Observation of Cooperative Protein Structural Dynamics of Homodimeric Hemoglobin from 100 ps to 10 ms with Pump-Probe X-ray Solution Scattering. *J. Am. Chem. Soc.* **2012**, *134*, 7001–7008.

(4) Levantino, M.; Schirò, G.; Lemke, H. T.; Cottone, G.; Glownia, J. M.; Zhu, D.; Chollet, M.; Ihee, H.; Cupane, A.; Cammarata, M. Ultrafast myoglobin structural dynamics observed with an X-ray free-electron laser. *Nat. Commun.* **2015**, *6*, 1–6.

(5) Aue, W. P.; Bartholdi, E.; Ernst, R. R. Two-dimensional spectroscopy. Application to nuclear magnetic resonance. *J. Chem. Phys.* **1976**, *64*, 2229–2246.

(6) Hamm, P.; Helbing, J.; Bredenbeck, J. Two-Dimensional Infrared Spectroscopy of Photoswitchable Peptides. *Annu. Rev. Phys. Chem.* **2008**, *59*, 291–317.

(7) Ghosh, A.; Ostrander, J. S.; Zanni, M. T. Watching Proteins Wiggle: Mapping Structures with Two-Dimensional Infrared Spectroscopy. *Chem. Rev.* **2017**, *117*, 10726–10759.

(8) Fuller, F. D.; Ogilvie, J. P. Experimental Implementations of Two-Dimensional Fourier Transform Electronic Spectroscopy. *Annu. Rev. Phys. Chem.* **2015**, *66*, 667–90.

(9) Oliver, T. A. A. Recent advances in multidimensional ultrafast spectroscopy. *R. Soc. open sci.* **2018**, *5*, 171425.

(10) Consani, C.; Auböck, G.; van Mourik, F.; Chergui, M. Ultrafast Tryptophan-to-Heme Electron Transfer in Myoglobins Revealed by UV 2D Spectroscopy. *Science.* **2013**, *339*, 1586–1589.

(11) Prokhorenko, V. I.; Picchiotti, A.; Pola, M.; Dijkstra, A. G.; Miller, R. J. New Insights into the Photophysics of DNA Nucleobases. *J. Phys. Chem. Lett.* **2016**, *7*, 4445–4450.

(12) Brixner, T.; Stenger, J.; Vaswani, H. M.; Cho, M.; Blankenship, R. E.; Fleming, G. R. Two-dimensional spectroscopy of electronic couplings in photosynthesis. *Nature* **2005**, *434*, 625–628.

(13) Georgakopoulou, S.; Frese, R. N.; Johnson, E.; Koolhaas, C.; Cogdell, R. J.; van Grondelle, R.; van der Zwan, G. Absorption and CD Spectroscopy and Modeling of Various LH2 Complexes from Purple Bacteria. *Biophys. J.* **2002**, *82*, 2184–2197.

(14) Fasman, G. D. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Springer US: Boston, MA, 1996.

(15) Dartigalongue, T.; Hache, F. Observation of sub-100ps conformational changes in photolyzed carbonmonoxy-myoglobin probed by time-resolved circular dichroism. *Chem. Phys. Lett.* **2005**, *415*, 313–316.

(16) Mangot, L.; Taupier, G.; Romeo, M.; Boeglin, A.; Cregut, O.; Honorat Dorkenoo, K. D. Broadband transient dichroism spectroscopy in chiral molecules. *Opt. Lett.* **2010**, *35*, 381–383.

(17) Trifonov, A.; Buchvarov, I.; Lohr, A.; Würthner, F.; Fiebig, T. Broadband femtosecond circular dichroism spectrometer with white-light polarization control. *Rev. Sci. Instrum.* **2010**, *81*, 43104.

(18) Hiramatsu, K.; Nagata, T. Communication: Broadband and ultrasensitive femtosecond time-resolved circular dichroism spectroscopy. *J. Chem. Phys.* **2015**, *143*, 121102.

(19) Oppermann, M.; Bauer, B.; Rossi, T.; Zinna, F.; Helbing, J.; Lacour, J.; Chergui, M. Ultrafast broadband circular dichroism in the deep ultraviolet. *Optica* **2019**, *6*, 56–60.

(20) Mendonça, L.; Steinbacher, A.; Bouganne, R.; Hache, F. Comparative Study of the Folding/Unfolding Dynamics of Poly(glutamic acid) in Light and Heavy Water. *J. Phys. Chem. B* **2014**, *118*, 5350–5356.

(21) Hollósi, M.; Kollát, E.; Kajtár, J.; Kajtár, M.; Fasman, G. D. Chiroptical Labeling of Folded Polypeptide Conformations: The Thioamide Probe. *Biopolymers* **1990**, *30*, 1061–1072.

(22) Baum, P.; Lochbrunner, S.; Riedle, E. Tunable sub-10-fs ultraviolet pulses generated by achromatic frequency doubling. *Opt. Lett.* **2004**, *29*, 1686-1688.

(23) Sifferlen, T.; Rueping, M.; Gademann, K.; Jaun, B.; Seebach, D. β-Thiopeptides: Synthesis, NMR Solution Structure, CD Spectra, and Photochemistry. *Helv. Chim. Acta* **1999**, *82*, 2067–2093.

(24) De Poli, M.; Clayden, J. Thionoglycine as a multifunctional spectroscopic reporter of screw-sense preference in helical foldamers. *Org. Biomol. Chem.* **2014**, *12*, 836–843.

(25) Miwa, J. H.; Pallivathucal, L.; Gowda, S.; Lee, K. E. Conformational Stability of Helical Peptides Containing a Thioamide Linkage. *Org. Lett.* **2002**, *4*, 4655–4657.

(26) Miwa, J. H.; Patel, a. K.; Vivatrat, N.; Popek, S. M.; Meyer, a. M. Compatibility of the Thioamide Functional Group with β-Sheet Secondary Structure: Incorporation of a Thioamide Linkage into a β-Hairpin Peptide. *Org. Lett.* **2001**, *3*, 3373–3375.

(27) Culik, R. M.; Jo, H.; Degrado, W. F.; Gai, F. Using Thioamides To Site-Specifically Interrogate the Dynamics of Hydrogen Bond Formation in β-Sheet Folding. *J. Am. Chem. Soc.* **2012**, *134*, 8026–8029.

(28) Batjargal, S.; Wang, Y. J.; Goldberg, J. M.; Wissner, R. F.; Petersson, E. J. Native Chemical Ligation of Thioamide-Containing Peptides: Development and Application to the Synthesis of Labeled α-Synuclein for Misfolding Studies. *J. Am. Chem. Soc.* **2012**, *134*, 9172–9182.

(29) Wang, Y. J.; Szantai-Kis, D. M.; Petersson, E. J. Semi-synthesis of thioamide containing proteins. *Org. Biomol. Chem.* **2015**, *13*, 5074–81.

(30) Huang, Y.; Ferrie, J. J.; Chen, X.; Zhang, Y.; Szantai-Kis, D. M.; Chenoweth, D. M.; Petersson, E. J. Electronic interactions of $i, i+1$ dithioamides: increased fluorescence quenching and evidence for n-to-π^* interactions. *Chem. Commun.* **2016**, *52*, 7798–7801.

(31) Walters, C. R.; Szantai-Kis, D. M.; Zhang, Y.; Reinert, Z. E.; Horne, W. S.; Chenoweth, D. M.; Petersson, E. J. The effects of thioamide backbone substitution on protein stability: a study in α-helical, β-sheet, and polyproline II helical contexts. *Chem. Sci.* **2017**, *8*, 2868–2877.

(32) Walters, C. R.; Ferrie, J. J.; Petersson, E. J. Dithioamide substitutions in proteins: effects on thermostability, peptide binding, and fluorescence quenching in calmodulin. *Chem. Commun.* **2018**, *54*, 1766–1769.

(33) Goldberg, J. M.; Batjargal, S.; Petersson, E. J. Thioamides as Fluorescence Quenching Probes: Minimalist Chromophores To Monitor Protein Dynamics. *J. Am. Chem. Soc.* **2010**, *132*, 14718–14720.

(34) Huang, R.; Kubelka, J.; Barber-Armstrong, W.; Silva, R. A. G. D.; Decatur, S. M.; Keiderling, T. A. Nature of Vibrational Coupling in Helical Peptides: An Isotopic Labeling Study. *J. Am. Chem. Soc.* **2004**, *126*, 2346–2354.

(35) Woutersen, S.; Hamm, P. Structure Determination of Trialanine in Water Using Polarization Sensitive Two-Dimensional Vibrational Spectroscopy. *J. Phys. Chem.* **2000**, *104*, 11316–11320.

(36) Cervetto, V.; Pfister, R.; Kolano, C.; Bregy, H.; Heimgartner, H.; Helbing, J. Coexistence of Hydrogen-Bonded Loop and Extended Tetrapeptide Conformations. *Chem. - A Eur. J.* **2007**, *13*, 9004–9011.

(37) Ihalainen, J. A.; Paoli, B.; Muff, S.; Backus, E. H. G.; Bredenbeck, J.; Woolley, G. A.; Caflisch, A.; Hamm, P. α-Helix folding in the presence of structural constraints. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 9588–9593.

(38) Helbing, J.; Bregy, H.; Bredenbeck, J.; Pfister, R.; Hamm, P.; Huber, R.; Wachtveitl, J.; De Vico, L.; Olivucci, M. A Fast Photoswitch for Minimally Perturbed Peptides: Investigation of the trans $\rightarrow$ cis Photoisomerization of *N*-Methylthioacetamide. *J. Am. Chem. Soc.* **2004**, *126*, 8823–8834.

(39) Satzger, H.; Root, C.; Gilch, P.; Zinth, W.; Wildemann, D.; Fischer, G. Photoswitchable Elements within a Peptide Backbone Ultrafast Spectroscopy of Thioxylated Amides. *J. Phys. Chem. B* **2005**, *109*, 4770–4775.

(40) Kirkwood, J. G. On the Theory of Optical Rotatory Power. *J. Chem. Phys.* **1937**, *5*, 479–491.

(41) Madison, V.; Schellman, J. Diamide Model for the Optical Activity of Collagen and Polyproline I and II. *Biopolymers* **1970**, *9*, 65–94.

(42) Kee Kang, Y.; Sook Park, H. Ab initio conformational study of N-acetyl-L-proline-N',N'-dimethylamide: a model for polyproline. *Biophys. Chem.* **2005**, *113*, 93–101.

(43) Newberry, R. W.; Vanveller, B.; Guzei, I. A.; Raines, R. T. $n \rightarrow \pi^*$ Interactions of Amides and Thioamides: Implications for Protein Stability. *J. Am. Chem. Soc.* **2013**, *135*, 7843–7846.

(44) Auböck, G.; Consani, C.; Monni, R.; Cannizzo, A.; Mourik, F. V. Femtosecond pump/supercontinuum-probe setup with 20 kHz repetition rate repetition rate. *Rev. Sci. Instrum.* **2012**, *093105*, 93105.

(45) Kjellberg, P.; Brüggemann, B.; Pullerits, T. Two-dimensional electronic spectroscopy of an excitonically coupled dimer. *Phys. Rev. B* **2006**, *74*, 1–9.

(46) Cervetto, V.; Bregy, H.; Hamm, P.; Helbing, J. Time-resolved IR spectroscopy of N-Methylthioacetamide: Trans $\rightarrow$ Cis Isomerization upon n-π^* and $\pi - \pi^*$ Excitation and Cis $\rightarrow$ Trans Photoreaction. *J. Phys. Chem. A* **2006**, *110*, 11473–11478.

(47) Xie, X.; Simon, J. D. Picosecond circular dichroism spectroscopy: a Jones matrix analysis. *J. Opt. Soc. Am. B* **1990**, *7*, 1673.

(48) Lewis, J. W.; Goldbeck, R. A.; Kliger, D. S.; Xie, X.; Dunn, R. C.; Simon, J. D. Time-Resolved Circular Dichroism Spectroscopy: Experiment, Theory, and Applications to Biological Systems. *J. Phys. Chem.* **1992**, *96*, 5243–5254.

(49) Donten, M. L.; Hassan, S.; Popp, A.; Halter, J.; Hauser, K.; Hamm, P. pH-Jump

Induced Leucine Zipper Folding beyond the Diffusion Limit. *J. Phys. Chem. B* **2015**, *119*, 1425–1432.

(50) Scheerer, D.; Chi, H.; McElheny, D.; Keiderling, T. A.; Hauser, K. Isotopically Site-Selected Dynamics of a Three-Stranded β-Sheet Peptide Detected with Temperature-Jump Infrared-Spectroscopy. *J. Phys. Chem. B* **2018**, *122*, 10445–10454.